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13. ABSTRACT ***(Maximum 200 Words)***

The regulation of prostate morphogenesis has been shown to be regulated by signaling molecules from the transforming growth factor beta family. The binding of the TGF-beta ligand to the cell surface receptors lead to the activation of a kinase activity in the TGF-beta receptors. The propagation of the signal through the cytoplasm is mediated by the Smad family of molecules. The project has examined the phosphorylation of Smad 2 and Smad 3 in the absence of TGF-beta 3 signaling. The Smad 2 molecule is specifically not phosphorylated in the null mutant while Smad 3 is unaffected. The effect appears to specific for the beta3 growth factor and rescue has not been shown with other TGF-beta family members. The null mutant represents a loss of function and in the absence of the growth factor no phosphorylation occurs, the effect appears to be growth factor specific. Current studies are examining the regulation of TGF-beta receptor kinase activation and the potential of genetic rescue with Smad 2 overexpression. These studies should provide information specific for signaling mechanisms essential to glandular morphogenesis and relevant to mechanisms associated with uncontrolled glandular neoplasms.

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Introduction:

Studies of the developmental biology of the prostate are likely to provide significant new understanding of the molecular mechanisms governing prostate development and malignant transformation. Previous studies and our preliminary data have demonstrated that transforming growth factor-β (TGF-β)-mediated signaling regulates prostate growth and morphogenesis. The signaling of the TGF-β molecules is regulated intracellularly by the Smad family of signal transduction molecules. Smad family members have both positive and negative effects on TGF-β signaling through interaction with the TGF-β cell surface receptors. The studies are focused on the molecular mechanism of TGF-β signal transduction and the role of these mechanisms in glandular morphogenesis.

Body:

The current research has focused on studies in Specific Aim 2 examining the relationship of TGF-β signaling and Smad 2 and Smad 3 transduction of that signal. Exciting new findings have permitted a focus on this aim through the use of TGF-β3 null mutant mice and the effects of this loss of function mutant on Smad signal transduction. These studies are still on-going and have not yet been submitted for publication, in part due to the transfer of the proposal when Dr. Zhao moved to a biotechnology industry position.

The TGF-β superfamily signal through a common set of two TGF-β receptors and this has lead to investigation of the different effects due to the three TGF-β ligands. TGF-β1, TGF-β2 and TGF-β3 all initially bind to TGF-β receptor type II (TβIIR). This leads to heterodimerization with TGF-β receptor type I (TβIR) and activation of the serine/threonine kinase activity of the heterodimer. The activated kinase receptor subsequently phosphorylates a pathway restricted Smad to transduce the signal through the cytoplasm. In the current studies the specific effects of TGF-β3 on the phosphorylation of Smad 2 have been examined through the use of a TGF-β3 null mutant loss of function model.

The findings have shown that the absence of TGF-β3 results in a specific absence of Smad2 phosphorylation. The absence of Smad2 phosphorylation inhibits the propagation of the TGF-β ligand signal. This effect of TGF-β3 is not rescued by either TGF-β1 or TGF-β2 suggesting that part of the differential effects of the different TGF-β ligands may be due to the activation of specific intracellular messengers. Since all three TGF-β ligands signal through a common set of 2 cell surface receptors this finding is quite unexpected and led to studies another pathway restricted Smad, Smad 3. There was no compensatory increase in Smad 3 and it was likewise not phosphorylated in the absence of TGF-β3. Current research is actively investigating whether TGF-β1 or TGF-β2 can rescue Smad 3 phosphorylation and thus propagate a different signaling pathway intracellularly. These findings are novel in that there is now the potential to examine the different TGF-β ligands with respect to intracellular markers that might explain the different signaling outcomes observed. The broad range of effects of the different TGF-β molecules might be explained by altered enzymatic activities of the common heterodimer receptors. Slight alterations in enzyme activity coordinated with a differing profile of pathway restricted Smad activation could lead to novel changes in gene expression subsequent to binding of a specific TGF-β ligand. This finding is of particular interest in that autocrine mechanisms of TGF-β signaling can restrict the activation of specific intracellular pathways in very limited populations of cells. Careful analysis of the temporo-spatial expression of the TGF-b ligands

and generation of activation of different Smad molecules is underway to more clearly detail the mechanism during glandular morphogenesis.

These findings have led to the initiation of a series of experiments designed to further achieve the aims stated in Specific Aim. These aims are based on the use of a genetically engineered strain of mice that over-express Smad 2 in epithelial cells. This over-expression represents an important opportunity to further characterize the role of Smad2 in the TGF-β3 signaling pathway by breeding to a TGF-β3 null mutant mouse strain. This model represents a unique opportunity to characterize the Smad2 mechanism since increased levels of Smad2 gene expression may overcome the loss of function of the TGF-β3 ligand. These genetically engineered tissues can be examined with respect to the effects of TGF-β1 and TGF-β2 on modification of the Smad2 and the consequent activation of TGF-β-type specific gene expression. At this time the lines have been crossed and several generations of mice generated. The process of defining genetic homogeneity is underway and in the next funding year these mice will be available to conduct unique opportunities not available by any other mechanism. The potential to develop a genetic rescue of the null mutant phenotype will provide novel insight into the mechanism overall and provide a means to determine whether specific types of TGF-β related interventions could be used to alter prostate growth and differentiation.

Future studies will be specifically addressed at answering the questions raised in the original proposal by achieving the specific aims. The present studies have advanced the understanding of the fundamental aspects of mechanism permitting more in depth examinations in the future. These types of studies will provide novel insight into the regulation of growth factor signaling and identify potential opportunities to intervene in the signaling events with therapeutic modalities.

Key Research Accomplishment:

- TGF-β3 null mutation specifically alters the phosphorylation of the Smad 2 intracellular mediator.
- Smad 3 does not compensate for Smad 2 in the null mutant background.
- Autocrine pathways of TGF-β3 signaling can lead to distinct activation of different pathway restricted Smad molecules.
- Generation of genetic rescue experiments will provide a unique means to analyze the mechanism in greater depth using Smad2 over-expression in a TGF-β3 null mutant background.

Reportable Outcomes:

At this time manuscripts have not been submitted for publication. Manuscripts are in preparation to present the effects on Smad2 phosphorylation of the TGF-β3 null mutant. The current studies will provide publishable in three areas; 1) TGF-β ligand specific activation of subsets of pathway restricted Smads through differential activation of receptor kinase activity; 2) Kinase activity of the TβIR-TβIIR complex following binding of different members of the TGF-β family; 3) Genetic rescue of TGF-β null mutations using specific overexpression of pathway restricted Smads and the relationship to the downstream activation of TGF-β regulated gene expression. All of these findings will have important implications for the basic understanding of

prostate growth and development and potential applications in eventual therapies to address altered patterns of prostate cell proliferation.

Conclusion:

The results to date have addressed a specific area of uncertainty regarding TGF-β signaling, differential effects of multiple growth factors following binding to a common set of cell surface receptors. The identification of altered modification of intracellular mediators will provide an excellent foundation for further investigation. The development of a novel genetic rescue of a TGF-β null mutation has generated a new approach to investigating the problem and new opportunities to address the specific research question.

Appendices:

None